HudsonAlpha
INSTITUTE FOR BIOTECHNOLOGY

The CSER (Clinical Sequencing Exploratory Research) analysis group at HudsonAlpha uses guidelines similar to the ACMG recommendations (Richards et al., Genet Med 2015, PMID: 25741868) to evaluate the clinical relevance of sequence variants. Variants are classified into one of five categories: Pathogenic, Likely Pathogenic, Unknown Significance ("VUS"), Likely Benign or Benign. VUSs may be sub-classified into "VUS – Lean Benign" and "VUS – Lean Pathogenic" as necessary.

Below is a brief summary of variant properties used to support any given designation, used as of September 2015. Consistent with ACMG rules, multiple lines of evidence are required to support assignments of pathogenic or likely pathogenic, while classifications of benign or likely benign require allele frequency estimates too high to be plausibly connected to disease and/or computational predictions that a variant is benign. Conflicting or ambiguous lines of evidence generally result in a designation of VUS.

Pathogenic

- Previous reports that a variant is pathogenic, where primary data is available for analysis, and the interpretation of pathogenicity is still supported (by allele frequencies, mechanism of action, other scientific literature, etc.).
- Variant clearly results in loss-of-function in a gene where loss-of-function is a known mechanism of disease.
- Variant is missense and computationally predicted to be damaging, in a gene where missense variation is a known mechanism of disease.
- Variant is de novo and predicted to be damaging or loss-of-function in a well-established dominant disease gene.
- Variant(s) are recessive or compound heterozygous (established via parental assessment), at frequencies low enough to be plausible for disease, predicted to be damaging, and present in a gene known to be recessively associated with disease.
- Patient's observed phenotype(s) is specifically consistent with those reported for the associated disease.

Likely Pathogenic

Variants that meet the criteria for Pathogenic, but have one of the following inconsistencies or uncertainties:

- Uncertainty regarding the molecular effect of the variant (e.g. a variant resulting in a premature stop near the end of a protein, affecting non-canonical splice sites, or affecting splicing in only a subset of transcript isoforms.)
- Patient's observed phenotype(s) are uncertain, undefined, or somewhat inconsistent with those reported for the associated disease.
- There is a discrepancy between the type of variation and known mechanism of disease.
- Inheritance status is unknown.

Unknown Significance (VUS)

- Variant is *de novo* and computationally predicted to be damaging.
- Variant(s) are very rare, predicted to be damaging, and exist in compound heterozygous or recessive states.
- Variant impacts a gene with a specific, plausible biological connection to disease.
- Variant impacts a gene predicted to be generally intolerant to variation.
- Any variant for which there are lines of evidence supporting both pathogenic and benign interpretations.

Likely Benign

- Variant impacts a gene predicted to be tolerant of variation.
- Variant has an allele frequency (in external or internal databases) near the observed frequency of disease.
- Variant is predicted computationally to be benign (e.g., synonymous replacements, lack of conservation, etc.).

Benign

- Variant has an allele frequency (in external or internal databases) that is higher than the observed frequency of disease.